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Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

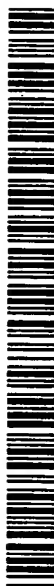
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10 May 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR PRODUCING RECOMBINANT PROTEINS

(57) Abstract: Methods are provided for producing and recovering recombinant proteins from plant tissue. The methods of the invention find use in the commercial processing of grains, particularly in the wet-milling of corn kernels. The methods involve steeping plant tissue and recovering the recombinant proteins in steep water. The methods additionally involve optimizing nucleic acid constructs and plants for the recovery of recombinant proteins from plant tissue.

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PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

SPRUILL, W., Murray
Alston & Bird LLP
Bank of America Plaza
101 South Tryon Street, Suite 4000
Charlotte, NC 28280-4000
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
14 September 2001 (14.09.01)

Applicant's or agent's file reference
41377/202011

International application No.
PCT/US00/26005

IMPORTANT NOTIFICATION

International filing date (day/month/year)
21 September 2000 (21.09.00)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address

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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

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Authorized officer

François BAECHLER

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | | |
|--|--|---|--|
| Applicant's or agent's file reference 41377/202011 | | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) | |
| International application No. PCT/US00/26005 | International filing date (day/month/year) 21/09/2000 | Priority date (day/month/year) 21/09/1999 | |
| International Patent Classification (IPC) or national classification and IPC C07K1/14 | | | |
| Applicant PRODIGENE, INC. et al. | | | |



1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

| | |
|---|--|
| Date of submission of the demand 19/04/2001 | Date of completion of this report 20.11.2001 |
| Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | Authorized officer Lopez Garcia, F Telephone No. +49 89 2399 2171  |

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US00/26005

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-30 as originally filed

Claims, No.:

1-47 as originally filed

Drawings, sheets:

1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US00/26005

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. N on-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☐ claims Nos. .

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-47 (partially).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes: Claims 3-5, 9, 19, 12, 14, 16, 23, 24, 27-29, 46, 47

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/US00/26005**

| | | | |
|-------------------------------|------|--------|--|
| | No: | Claims | 1,2, 6-8, 11, 13, 15, 17, 18-22, 25, 26, 30-45 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-47 |
| Industrial applicability (IA) | Yes: | Claims | 1-47 |
| | No: | Claims | |

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

s separate sheet

Re Items I & III

Basis of the opinion & Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

A partial search has been carried out (see ISA 210) where the subject-matter of claims 1-47 has been restricted to the supported/disclosed claimed methods, namely the methods for recovering aprotinin from transgenic plants as described in examples 1 and 2 and tables 1 and 2 on pages 26 to 29 of the description. Therefore, the exam will be restricted also to these methods (Rule 66.1(e) PCT).

The Applicant should limit the application to the invention searched and excise those parts of the application not relating thereto.

R Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 97 17453 A

D2: WO 97 04123 A

D3: EP-A-0 321 004

D4: WO 94 08027 A

2. The application discloses a method for producing aprotinin from transgenic plant tissues by providing a transgenic plant expressing said protein, taking the tissue where said aprotinin is accumulated and recovering said protein by steeping said tissue with a steeping solution, ie mixing the tissue with water (see p. 4, l. 22 of the present application).

3. Novelty of the searched subject-matter.

D1 discloses a method for producing aprotinin from transgenic plants (see examples 1 and 2 at p. 16 and 17, respectively), where the aprotinin is extracted by conventional methods (p. 13, l. 33-36 and example 3). Said methods are

described by Heney and Orr, Anal. Biochem, 114, 92-96 (D5, provided). The method of Heney and Orr consists in diluting the protein source with water and ammonium sulfate and extracting the protein from said water (see p. 93, LH col. paragraph 4). Therefore, the subject-matter of claims **1, 2, 6-8, 11, 13, 15, 17, 18-22, 25, 26, 30-45** is not novel (**Art. 33(2) PCT**), insofar as the search subject-matter is concerned.

Since D2-D4 do not disclose any extraction process of aprotinin, said documents are not relevant for the novelty of the searched claimed-matter.

4. Despite the search has been restricted (see above), there has been found documents relevant for the novelty of the broad scope of the claims. Just as example:

D2 provides transgenic plants that express collagen compounds which are recovered from transgenic plant corns by steeping said tissues and recovering said collagen compounds from the steep water (p. 34, l. 6-p. 35, l. 6). As mentioned in D2 (p. 3, l. 14- p. 4, l. 22), transgenic plants have been used to produce foreign biological proteins. The subject-matter of claims **1, 6-8, 12, 13, 15, 17, 19, 21, 22, 25, 30-34, 36-42, 44 and 45** is not novel (**Art. 33(2) PCT**).

4. At present, the subject-matter of independent claims **1, 25 and 36** is not novel. Nor are the dependent claims **2, 6-8, 11, 13, 15, 17, 18-22, 26, 30-35, 37-45**.

The subject-matter of claims **3-5, 9, 10, 12, 14, 16, 23, 24, 27-29, 46, 47** does not seem to bring any inventive merit over the content of D1 alone or combined with the content of D3 and D4, when providing alternative processes for preparing aprotinin.

D3 discloses that proteins can be obtained by steeping corn or sorghum kernels in warm water containing sulfur. Advantages are that water soften the kernels and water soluble substances pass into the steep water and that the sulfur dioxide breaks the cell wall and prevents growth of microorganisms (see p. 2, l. 1-8).

D4 discloses that the wet-milling-based process is one of the usual processes for

preparing products from corn kernels. This process consists in breaking the bonds between starch and protein in the endosperm by steeping the corn with water, sulfur dioxide and lactic acid. The corn steep liquor contains about 21% proteins (p. 1, l. 15 - p. 2, l. 1).

Advantages of steeping recovering processes to obtain products (in particular proteins) from plant tissues are known from D3 and D4. Despite the methods of D3 and D4 are applied to extract plant proteins, the fact that the proteins expressed for the plant is a foreign protein is not relevant for the purification process. Therefore, the skilled person when face with the problem posed above, will use the steeping processes of D3 and D4 in the process of D1, arriving then to the claimed process. Therefore, the subject-matter of claims 1-47 is considered as not inventive (**Art. 33(3) PCT**).

Since the nature of the protein to be purified is important when elaborating a extraction/purification process, it is doubtful that the claimed process can be applied to any plant and to any protein to be purified.

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D5 is not mentioned in the description, nor are these documents identified therein.
2. Expressions as "incorporated by reference" (p. 12, l. 3, p 14, l. 8, p. 17, l. 22, p. 18, l. 2, p. 21, l. 18, l. 28, p. 22, l. 30, p. 23, l. 33, p. 24, l. 26, p. 30, l. 1-6) are not allowable (see Guidelines PCT II-4.17) since the patent specifications should, regarding the essential features of the invention, be self-contained, that is, capable of being understood without reference to any other document.

R Item VIII

Certain observations on the international application

1. The subject-matter of present claim 1 is directed to any extraction method of any

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/26005

recombinant enzyme from any plant and plant tissue since it is indicated on p. 5, l. 13-17 of the description that the seeds can be broken before the steeping.

Without any limitation of the process, the subject-matter as it is defined places an undue burden on the skilled person wishing to carry out the invention. Therefore, the subject-matter of independent claims 1, 25, and 36 does not meet the requirements of Art. 6 PCT.

2. The term "about" used in claim 2-5, 26-29, in relation to numerical values renders unclear the scope of said claim and should be deleted from them (Art. 6 PCT).
3. The vague and imprecise statements in the description on page 30, l. 7-10 and on p. 26, l. 1-2 and the expressions "and the like" and "not limited to" (p. 3, l. 31, p. 4, l. 12, 21, 23, p. 5, l. 5, 15, 17, 19, 24, p. 7, l. 22, p. 8, l. 3, 6, 11, p. p. 9, l. 2, p. 12, l. 30, p. 13, l. 8, 14, 20, p. 14, l. 20, 23, p. 17, l. 6, 24, p. 18, l. 16, 23, 27, 28, p. 20, l. 19, 25, p. 21, l. 8, 31, 32, 32, p. 22, l. 15, p. 24, l. 5, 27, p. 25, l. 5, 29) imply that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).